# Ultrathin-Layer Chromatography

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## Abstract

**The development of ultrathin-layer silica gel plates with a monolithic structure opens up a new dimension in thin-layer chromatography (TLC). The very small layer thickness of approximately 10 µm and the absence of any kind of binder in combination with the framework of this stationary phase lead to new and improved properties of these ultrathin-layer chromatographic (UTLC) silica gel plates compared with conventional TLC and high-performance (HP) TLC precoated layers. First of all, the advantages of the UTLC plates are the very short migration distances and, in combination with this, the short development times as well as the very low consumption of solvents as the mobile phase in connection with high sensitivity. The separations of amino acids, pesticides, pharmaceutically active ingredients, phenols, and plasticizers effectively demonstrate the possibilities of the new ultrathin-layer silica gel plates. Furthermore, a comparison of UTLC, HP-TLC, and TLC concerning retention behavior, efficiency, detection limits, migration times, and solvent consumption is performed effectively by the separation of caffeine and paracetamol.**

## Introduction

In the past, the requirement of continual improvements in the efficiency of stationary phases in thin-layer chromatography (TLC) was mainly fulfilled by the following developments: (*a*) standardization of silica gels in 1958 by E. Stahl (1), (*b*) introduction of precoated TLC silica gel plates in 1966, (*c*) development of high-performance (HP) TLC layers in 1975, (*d*) surface modifications of silica gels for TLC and HP-TLC in 1978, and (*e*) application of spherical adsorbents for HP-TLC in 1995.

Another general trend in modern analytical methods besides the increase of efficiency is the miniaturization. In order to accomplish both demands, a new generation of precoated silica gel layers for planar chromatography have been developed: the ultra-thin layers (2). These new ultrathin-layer chromatographic (UTLC) silica gel plates with a layer thickness of approximately only 10 µm (the usual layer thickness in TLC is 250 µm) consist in contrast to normal TLC and HP-TLC layers not of separate sorbent particles, rather they have a monolithic structure. This monolithic structure, which no longer needs any kind of a binder in the layer, is formed by the hydrolytic polycondensation of a liquid film of an alkoxysiloxane on a glass plate. The manufacturing process described leads to a higher purity of the silica gel layer compared with the silica gels normally used in TLC and HP-TLC.

By controlling the production parameters a defined pore structure is generated in the monolithic silica gel layer. This pore structure consists of meso- and macropores, which together with the obtained specific pore volume and specific surface area determine the chromatographic behavior of the monolithic silica gel sorbent material. Table I gives a survey of the physical properties of this silica gel.

## Experimental

Because of the very small layer thickness and the special structure and properties of the UTLC layers, adequate chromatographic conditions have to be used that differ clearly from the usual conditions in TLC and HP-TLC. A survey of these conditions is listed in Table II.

In order to demonstrate the properties and possibilities of the UTLC silica gel plates, separations of amino acids, pharmaceutically active ingredients, phenols, and plasticizers were performed.

### Separation of amino acids

For the study of amino acids, the sample substances used were

**Table I. Physical Properties of the UTLC Silica Gel Plates**

| | |
|---|---|
| Mesopores | 3–4-nm pore diameter |
| Macropores | 1–2-µm pore diameter |
| Specific surface area | ~ 350 m2/g |
| Specific pore volume (mesopores) | ~ 0.3 mL/g |
| Layer thickness | ~ 10 µm |

**Table II. Chromatographic Conditions in UTLC**

| **Sample application volume** | **5–20 nL (spotwise application) up to 200 nL (bandwise application)** |
|---|---|
| Migration distance | 1–3 cm |
| Migration time | 1–6 min |
| Consumption of mobile phase | 1–4 mL |

glycylproline, D-norvaline, and tryptophan. The application volume was 10 nL (~0.05% solution in methanol). The spotting device was a Hamilton syringe (0.5 µL total volume). The mobile phase used was water–acetonitrile–glacial acetic acid (10:3:57.5, v/v/v). The migration distance was 2 cm in a normal chamber with chamber saturation, and the migration time was 210 s. Detection was determined by staining with ninhydrin. Documentation was performed by the use of video documentation (ProViDoc, DESAGA, Wiesloch, Germany).

**Separation of pesticides**

The sample substances used were metoxuron, desisopropylatrazin, cyanazin, and trifluralin. The application volume was 20 nL (0.1% solution in acetonitrile). The Sample Applicator ATS 4 (Camag, Muttenz, Switzerland) was used as the spotting device. The mobile phase used was petroleum benzene–acetone (70:30, v/v). The migration distance was 2 cm in a normal chamber with chamber saturation, and the migration time was 260 s. Evaluation was performed with a diode-array spectrophotometer (J&M, Aalen, Germany) at UV 200 nm.

**Separation of pharmaceutically active ingredients**

The sample substances used were bromazepam, diazepam, and parazepam. The application volume was 10 nL (0.2% solution in toluene). A Hamilton syringe (0.5 µL total volume) was used as the spotting device. The mobile phase used was *n*-hexane–ethyl acetate (50:50, v/v). The migration distance was 2 cm in a normal chamber with chamber saturation, and the migration time was 250 s. Evaluation was performed with a TLC Scanner II (Camag) at UV 254 nm.

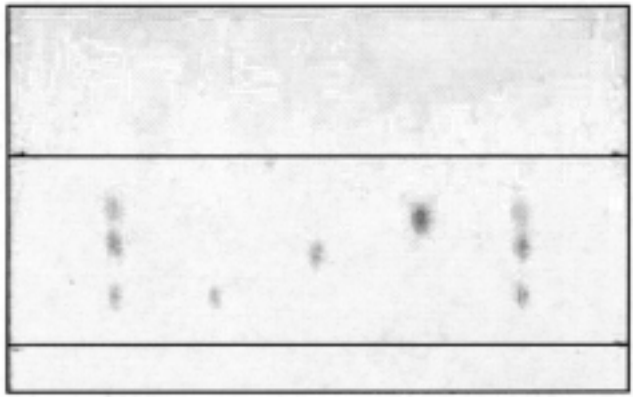

**Figure 1.** Separation of amino acids.

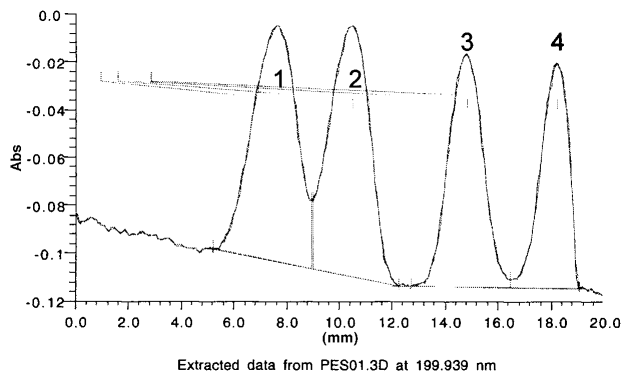


**Figure 2.** Separation of pesticides: (1) metoxuron, (2) desisopropylatrazin, (3) cyanazin, and (4) trifluralin.

**Separation of phenols**

The sample substances used were 4-aminophenol, 2-aminophenol, 4-chlorophenol, and 2,5-dinitrophenol. The application volume was 10 nL (0.1% solution in acetonitrile). A Sample Applicator ATS 4 (Camag) was used as the spotting device. The mobile phase used was toluene–chloroform–methanol (80:10:10, v/v/v). The migration distance was 2 cm in a normal chamber with chamber saturation, and the migration time was 240 s. Evaluation was performed with a diode-array spectrophotometer (J&M) at UV 200 nm.

**Separation of plasticizers**

The sample substances used were dimethyl phthalate, diethyl phthalate, and dibutyl phthalate. The application volume was 10 nL (0.4% solution in methanol). A Hamilton syringe (0.5 µL total

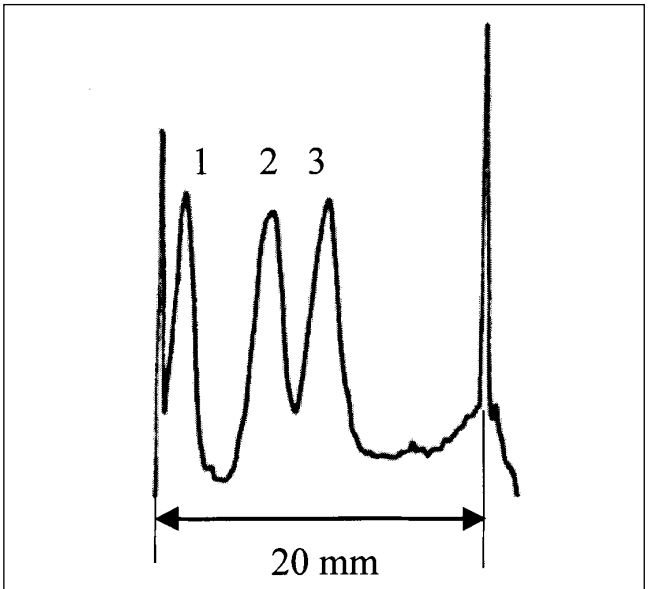


**Figure 3.** Separation of pharmaceutically active ingredients: (1) bromazepam, (2) diazepam, and (3) parazepam.

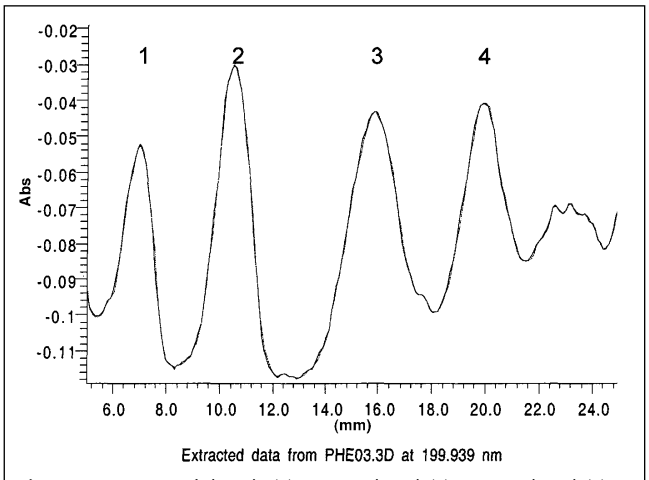


**Figure 4.** Separation of phenols: (1) 4-aminophenol, (2) 2-aminophenol, (3) 4-chlorophenol, and (4) 2,5-dinitrophenol.

volume) was used as the spotting device. The mobile phase used was petroleum benzine (40–60°)–acetone (90:10, v/v). The migration distance was 2 cm in a normal chamber with chamber saturation, and the migration time was 240 s. Evaluation was performed with a diode-array spectrophotometer (J&M) at UV 234 nm.

**Comparison of UTLC, HP-TLC, and TLC**

The retention behavior, efficiency, migration times, detection limits, and solvent consumption of UTLC plates was compared with the corresponding data of HP-TLC and TLC silica gel 60 precoated layers by the separation of caffeine and paracetamol.

For the separation of caffeine and paracetamol, the following conditions were used. For plates, UTLC silica gel plates, HP-TLC silica gel 60 plates, and TLC silica gel 60 plates were used. The sample substances were caffeine and paracetamol. The application volumes were 10 nL for UTLC, 100 nL for HP-TLC, and 2 µL for TLC. The Sample Applicator ATS 4 (Camag) was used as the spotting device. The mobile phase used was ethyl acetate–methanol (99:1, v/v). The migration distances were 1 cm in a normal chamber with saturation for UTLC, 5 cm in a normal chamber with saturation for HP-TLC, and 10 cm in a normal chamber with saturation for TLC. Evaluation was performed with a TLC Scanner II (Camag) at UV 200 nm.

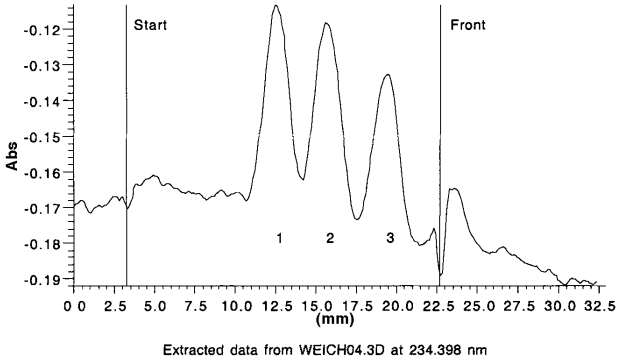


**Figure 5.** Separation of plasticizers: (1) dimethyl phthalate, (2) diethyl phthalate, and (3) dibutyl phthalate.

**Table III. Comparison of Detection Limits, Migration Times, and Solvent Consumption in UTLC, HP-TLC, and TLC**

| Type of layer | Detection limit for caffeine (ng) | Migration time (s) | Solvent consumption (mL) |
|---|---|---|---|
| UTLC | 1 | 105 | 3 |
| HP-TLC | 10 | 385 | 40 |
| TLC | 25 | 935 | 100 |

**Table IV. Comparison of Retention Data, Plate Heights, and Resolution in UTLC, HP-TLC, and TLC**

| Type of layer | hRf | | Plate height (µm) | | Resolution |
|---|---|---|---|---|---|
| | Caffeine | Paracetamol | Caffeine | Paracetamol | |
| UTLC | 15.0 | 45.0 | 104 | 80 | 2.1 |
| HP-TLC | 13.3 | 36.7 | 50 | 30 | 4.6 |
| TLC | 10.7 | 32.4 | 172 | 52 | 4.1 |

## Results and Discussion

The chromatograms obtained on the UTLC silica gel plates are shown in Figure 1 (amino acids), Figure 2 (pesticides), Figure 3 (pharmaceutically active ingredients), Figure 4 (phenols), and Figure 5 (plasticizers).

All of these separations were examples for the efficiency of the monolithic UTLC silica gel plates, especially with regard to the sensitivity, speed, and consumption of mobile phases.

The results of the comparison of UTLC, HP-TLC, and TLC are listed in Tables III and IV.

The data in Table III point out the clear improvement of UTLC compared with HP-TLC and TLC with regard to detection limits, migration times, and solvent consumption.

The higher hRf values on UTLC layers compared with HP-TLC and TLC silica gel 60 plates can be explained by the lower specific surface area of the UTLC plates (350 m?/g instead of 500 m?/g at TLC and HP-TLC).

The rather high plate heights on the UTLC plate can be explained by the up to now given insufficiency of the application and evaluation technique in combination with the very short migration distance influencing the result in the calculation formula of plate heights.

The lower value of the resolution in the case of UTLC can additionally be explained by the drastically reduced separation distance in this case.

A precondition to exhaust the possibilities of the UTLC silica gel plates was the supply of adequate and efficient equipment for sample application, development, and evaluation of this new generation of precoated layers.

## Conclusion

The introduction of monolithic UTLC silica gel plates leads to the further development of planar chromatography in the direction of miniaturization and a general increase in the efficiency of this analytical method. In particular, the drastic reduction in migration times and solvent consumption in combination with a distinct improvement in sensitivity was emphasized in this study.

## References

1. E. Stahl. *Chem.-Ztg.* **82:** 323 (1958).
2. H.E. Hauck, O. Bund, W. Fischer, and M. Schulz. *J. Planar Chromatogr.* **14:** 234 (2001).

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